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ORIGINAL ARTICLE

Correlation between Peripheral Blood T-cell Profiles and Clinical and Inflammatory Parameters in Stable COPD

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ABSTRACT

Background: Recent studies suggest that Tc1/Tc2 imbalances are implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD). The purpose of this study was to clarify the relationship between peripheral blood T-cell profiles and pulmonary function or inflammatory parameters.

Methods: Thirty-one patients with stable COPD (median age 70 years, 30 males, 15 current smokers and 16 ex-smokers) and 30 healthy control subjects were enrolled in this study. The subjects underwent blood tests, exhaled nitric oxide (eNO) measurement, pulmonary function tests, and sputum induction. Tc1/Tc2 and Th1/Th2 were determined by analyzing intracellular cytokine staining for IFN-γ and IL-4 in peripheral blood $CD8^+$ and $CD4^+$ T cells using flow cytometry after stimulation with phorbol 12-myristate 13-acetate and ionomycin.

Results: There was a significantly increased proportion of IFN-γ-producing and IL-4-producing $CD8^+$ T cells in patients with COPD compared with control subjects (median [IQR] 73.6% [63.9%-80.7%] vs 62.0% [45.6%-73.8%], $p = 0.004$; and 2.6% [1.1%-6.9%] vs 1.1% [0.6%-2.2%], $p = 0.002$, respectively). In addition, the proportion of IFN-γ-producing $CD4^+$ T cells was significantly higher in patients with COPD compared with control subjects (25.7% [21.2%-38.0%] vs 22.8% [15.6%-29.2%], $p = 0.027$). The proportion of IFN-γ-producing $CD8^+$ T cells was correlated negatively with single-breath carbon monoxide transfer coefficient (Kco) ($\rho = -0.45$, $p = 0.033$) and positively with eNO ($\rho = 0.50$, $p = 0.012$). The proportion of IL-4-producing $CD8^+$ T cells was positively correlated with body mass index ($\rho = 0.42$, $p = 0.023$) and Kco ($\rho = 0.47$, $p = 0.026$).

Conclusions: It is suggested that Tc1 cells have a detrimental role and that Tc2 cells have a protective role in disease progression.

KEY WORDS

chronic obstructive pulmonary disease, correlation, exhaled nitric oxide, Tc1/Tc2, transfer coefficient

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammation in the respiratory tract with neutrophils, macrophages, and T lymphocytes.[1,2] It is known that there is a greater increase in $CD8^+$ than $CD4^+$ T cells in the airway wall and lung parenchyma.[3,4] It is suggested that the majority of T cells in COPD airways are of Tc1 (IFN-γ producing) rather than Tc2 (IL-4-producing) subtypes by immunohistochemical study.[5]

The flow cytometric method for examining intracellular cytokine production at the single-cell level is sensitive and does not need purification of peripheral blood mononuclear cells, which might modify the cell activation.[6-9] Very few investigators have studied the balance of Tc1 and Tc2 cells in peripheral blood from patients with COPD using the flow cytometric method. No increase or decrease of IFN-γ-producing or IL-4-producing $CD8^+$ T cells has been reported,[10] whereas an increased proportion of IFN-γ-producing $CD4^+$, not $CD8^+$, T cells has been found.[11] Recent studies have investigated cytokine production in bronchoalveolar lavage or induced sputum T cells,

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Table 1 Characteristics of the study population †

	Patients with COPD (n = 31)	Healthy control subjects (n = 30)
Age, years	70 (62-75)	67 (59-72)
Sex (male/female)	30/1	27/3
BMI, kg/m^2	21.5 (17.6-25.2)	22.5 (20.9-24.8)
Smoking history		
current/ex-smoker	15/16	14/16
pack-years	51 (40-79)	47 (38-75)
Severity (GOLD)		
mild/moderate/severe	11/12/8	Not applicable
FEV_1, % predicted	63.5 (49.3-95.7) ‡	104.6 (96.6-113.9)
FEV_1/FVC, %	51.2 (43.4-64.9) ‡	83.3 (80.2-86.3)
TLC, % predicted	114.3 (102.3-120.0)	106.6 (96.9-112.8)
FRC, % predicted	101.6 (94.1-111.6)	97.5 (86.1-104.2)
RV, % predicted	162.8 (132.3-183.1) ‡	109.0 (84.5-123.0)
Kco, % predicted	57.4 (39.6-68.6) ‡	93.8 (90.4-97.0)
Sputum neutrophils, %	58.3 (34.7-74.5)	44.1 (25.5-57.6)
Exhaled NO, ppb	26.3 (21.3-43.6)	31.4 (23.3-37.5)
Serum high sensitivity CRP, ng/ml	1130 (397-2580) ‡	392 (235-881)

BMI, body mass index; GOLD, Global Initiative for Chronic Obstructive Lung Disease; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; Kco, carbon monoxide transfer coefficient; NO, nitric oxide; CRP, C-reactive protein.

† Data are expressed as the median (IQR) or No.

‡ $P < 0.05$ compared with the control group.

raising the possibility that Tc2, rather than Tc1 cells might be predominant and that pulmonary T lymphocyte profiles might be different from those of peripheral blood[10,12,13]; thus, a consensus has not been established.

There is increasing evidence of systemic as well as local inflammation in patients with COPD. Induced sputum neutrophil counts and exhaled nitric oxide (NO) are useful markers of airway inflammation,[14-16] while recent studies suggest that high sensitivity C-reactive protein (CRP) levels may be systemic markers of the inflammatory process in COPD.[17,18] Very few studies have assessed the relationship between T cell profiles and clinical parameters, including pulmonary function tests. To our knowledge, no relationship with systemic or airway inflammation has been reported.

The purpose of this study was to clarify the balances of peripheral blood Tc1/Tc2 and Th1/Th2 in patients with COPD compared with those in healthy subjects, and the relationship between T-cell profiles and pulmonary function or inflammatory parameters, including induced sputum neutrophils, exhaled nitric oxide, and serum high sensitivity CRP.

METHODS

SUBJECTS

Thirty-one patients with stable COPD and 30 healthy control subjects were enrolled in this study (Table 1). These consecutive patients with COPD first visited Shizuoka General Hospital from May 2005 to October 2007. They satisfied the definition of the Global Initiative for Chronic Obstructive Lung Disease (GOLD)[1] and the severity was classified accordingly. The postbronchodilator forced expiratory volume in 1 second (FEV_1) and the ratio of FEV_1 to forced vital capacity (FVC) were determined 30 minutes after inhalation of 200 μg of salbutamol. All patients had a reversibility of less than 12% in FEV_1 before and 30 minutes after inhalation of salbutamol, used bronchodilator therapy only, and none had received oral or inhaled corticosteroids. Patients with COPD had no exacerbations, defined as increased dyspnea associated with a change in the quality and quantity of sputum, for at least one month before the first visit.

All subjects of both groups were current or ex-smokers. They were excluded from the study if they (1) had any history of asthma; (2) had atopy, defined by positive specific IgE antibodies to at least one inhalant allergen, including house dust mites, grass pollens, molds, cockroaches, cats, or dogs (CAP system, Pharmacia, Uppsala, Sweden); (3) had any acute viral infections within at least one month before the study; and (4) had inflammatory disease in which high CRP levels have been reported. The protocols were approved by the local ethics committee and informed consent was obtained from all patients prior to the study.

STUDY DESIGN

The subjects underwent blood tests, exhaled NO measurement, pulmonary function tests, and sputum

induction. Exhaled NO levels were measured by using the online method with an NO analyzer (Sievers NOA 280i, Sievers, Boulder, Colorado, USA) according to the American Thoracic Society/European Respiratory Society recommendations.[19] Spirometry, lung volumes, and transfer coefficient (Kco) were determined using computerized equipment (model CHESTAC-33; CHEST MI, Inc, Tokyo, Japan) according to the recommendations of the American Thoracic Society.[20,21] FEV_1 and FVC were expressed as a percentage of predicted values according to the formula of the Japanese Respiratory Society.[22] Total lung capacity (TLC), functional residual capacity (FRC), and residual volume (RV) were expressed as a percentage of predicted values according to the formula of Nishida.[23] Kco for carbon monoxide was measured by the single-breath method according to the guidelines of the Japanese Respiratory Society.[22] Results were corrected by haemoglobin concentration and expressed as a percentage of predicted values according to the formula of Burrows.[24] Sputum induction was performed according to the method described previously.[25] Serum high sensitivity CRP levels were measured by the latex-enhanced immunonephelometric method (N Latex CRP II, Dade Behring, Deerfield, Illinois, USA). The minimal detectable concentration of this assay system was 0.10 ng/ml.[26]

PREPARATION OF PERIPHERAL BLOOD CELLS

Ten milliliters of heparinized blood was collected from each patient or control subject and diluted 1 : 20 with a culture medium consisting of RPMI-1640.

STIMULATION OF CELLS AND INTRACELLULAR CYTOKINE STAINING

One milliliter of peripheral blood cells diluted 1 : 20 in culture medium was stimulated with phorbol 12-myristate 13-acetate (PMA; 25 ng/ml, Sigma, St Louis, Missouri, USA) and ionomycin (1 μg/ml, Sigma) in the presence of brefeldin-A (10 μg/ml, Sigma) for 4 hours at 37℃.

After stimulation with PMA/ionomycin, the cells were incubated with either peridin chlorophyll protein (PerCP)-conjugated anti-CD8 or anti-CD4 antibody (Becton Dickinson, San Jose, California, USA) for 15 minutes at room temperature (RT). In the preparation of peripheral blood cells, erythrocytes were lysed by adding FACS Lysing Solution (Beckton Dickinson). After centrifugation, the cells were washed with 0.1% BSA/PBS and subsequently incubated in FACS Permeabilizing Solution (Beckton Dickinson) for 10 minutes at RT. After washing with 0.1% BSA/PBS, the permeabilized cells were then incubated with fluorescein isothiocyanate (FITC)-conjugated anti-IFN-γ and phycoerythrin (PE)-conjugated anti-IL-4 antibodies for 30 minutes at RT. After staining, they were washed with 0.1% BSA/PBS, fixed in 1% paraformaldehyde, and analyzed. Negative controls consisted of unstimulated cells. We confirmed that the viability of the cells freshly obtained from peripheral blood was greater than 97% by using trypan blue exclusion. After PMA/ionomycin activation, the viability of each cell population was greater than 95%.

FLOW CYTOMETRIC ANALYSIS

The samples were analyzed on a FACScan flow cytometer (Beckton Dickinson) equipped with a 15-mW argon ion laser and appropriate filters for FITC (530 nm), PE (585 nm), and PerCP (>650 nm) by using the Cell Quest Software (Beckton Dickinson). Lymphocytes were first gated on the basis of forward and side light scatter. Subsequently, within this gated area, a second gate was further selected. The following analyses were done on cells in the second gate. For 3-colour analysis, we measured the number of positive cells for anti-IFN-γ (FITC), anti-IL-4 (PE), or both antibodies simultaneously in gated cell populations that were stained with either anti-CD8 or anti-CD4 antibody (PerCP). The number of positive cells for each cytokine was expressed as a percentage of $CD8^+$ or $CD4^+$ T cells. In all experiments, parallel incubations were performed with FITC- or PE-conjugated irrelevant antibodies matched for the isotypes of the anticytokine antibodies. The cutoff level for the definition of positive cells was thus set so that less than 1% of irrelevant antibody-stained cells was positive.

STATISTICAL ANALYSIS

All data are expressed as medians with interquartile ranges. Comparisons between groups were made with the Mann-Whitney U test or the Kruskal-Wallis test. Correlations between variables were done by using the Spearman rank correlation coefficient. Stat View Version 5.0 (SAS Institute, Cary, North Carolina, USA) was used for the statistical calculations. A P value of less than 0.05 was considered significant, and all tests were 2 sided.

RESULTS

CHARACTERISTICS OF THE STUDY POPULATION

As shown in Table 1, patients with COPD had lower FEV_1, FEV_1/FVC, and Kco, and higher RV and serum high sensitivity CRP levels compared with control subjects. There was no difference in exhaled NO or sputum neutrophil counts between COPD and healthy subjects. Table 2 shows the comparison of characteristics according to the severity of COPD. There were significant differences in body mass index, FEV_1, FEV_1/FVC, and RV; however, no significant difference was found in inflammatory parameters among mild, moderate, and severe patients.

Table 2 Comparison of characteristics according to the severity of COPD †

	Mild (n = 11)	Moderate (n = 12)	Severe (n = 8)	p value
Age, years	72 (65-76)	70 (65-74)	63 (60-71)	0.163
Sex (male/female)	11/0	11/1	8/0	0.441
BMI, kg/m^2	25.2 (21.5-26.5)	19.4 (17.7-21.6)	19.6 (16.3-23.8)	0.041
Smoking history				
current/ex-smoker	6/5	6/6	5/3	0.859
pack-years	48 (43-54)	51 (40-59)	83 (37-112)	0.264
FEV_1, % predicted	105.8 (95.2-118.9)	60.6 (57.7-67.9)	39.8 (38.5-43.4)	<0.001
FEV_1/FVC, %	66.0 (60.0-67.6)	49.1 (47.0-53.7)	37.0 (36.2-40.3)	<0.001
TLC, % predicted	115.8 (106.1-124.4)	114.7 (101.5-122.6)	107.7 (101.9-118.5)	0.741
FRC, % predicted	94.5 (93.6-108.0)	100.5 (94.1-114.1)	111.6 (99.5-115.9)	0.275
RV, % predicted	129.7 (113.7-138.0)	152.8 (137.0-181.4)	183.1 (171.1-191.5)	0.022
Kco, % predicted	69.1 (57.4-78.3)	51.2 (36.7-67.0)	49.0 (40.9-55.9)	0.090
Sputum neutrophils, %	58.3 (31.2-85.8)	58.8 (32.7-71.2)	58.9 (54.5-67.0)	0.979
Exhaled NO, ppb	23.4 (19.8-43.8)	24.2 (20.7-34.3)	30.0 (25.9-47.2)	0.375
Serum high sensitivity CRP, ng/ml	2530 (581-4780)	1080 (380-2263)	541 (369-1131)	0.158
IFN-γ-producing CD8$^+$ T cells, %	73.6 (63.9-80.7)	71.5 (62.0-79.1)	80.8 (57.1-84.4)	0.684
IL-4-producing CD8$^+$ T cells, %	2.6 (1.1-6.9)	2.5 (1.2-4.1)	2.8 (0.9-5.1)	0.998
IFN-γ-producing/IL-4-producing CD8$^+$ T cells	29.3 (9.3-117.9)	30.5 (16.4-71.6)	32.4 (11.8-99.3)	0.999
IFN-γ-producing CD4$^+$ T cells, %	25.7 (21.2-38.0)	28.2 (25.7-35.1)	25.0 (18.1-31.2)	0.429
IL-4-producing CD4$^+$ T cells, %	2.4 (1.5-3.4)	2.1 (1.4-3.4)	4.0 (2.9-5.5)	0.120
IFN-γ-producing/IL-4-producing CD4$^+$ T cells	13.4 (7.9-17.5)	14.9 (7.9-20.8)	10.1 (6.5-7.7)	0.513

BMI, body mass index; GOLD, Global Initiative for Chronic Obstructive Lung Disease; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; Kco, carbon monoxide transfer coefficient; NO, nitric oxide; CRP, C-reactive protein.

† Data are expressed as the median (IQR) or No.

PERIPHERAL BLOOD T-CELL PROFILES: COMPARISON BETWEEN PATIENTS WITH COPD AND HEALTHY SUBJECTS

Under unstimulated conditions, we could find no cytokine-expressing cells in the CD8$^+$ or CD4$^+$ T-cell population of peripheral blood cells in patients or healthy control subjects.

According to the response to PMA/ionomycin stimulation, each CD8$^+$ or CD4$^+$ T-cell was divided into 4 subsets: IFN-γ (+) IL-4 (-), type 1 cell; IFN-γ (-) IL-4 (+), type 2 cell; IFN-γ (+) IL-4 (+), type 0 cell; and IFN-γ (-) IL-4 (-), naive cell. We studied the proportion of type 1 and type 2 cells and the ratio of type 1/type 2 cells.

The proportion of IFN-γ-producing and IL-4-producing CD8$^+$ T cells were significantly higher in patients with COPD than in healthy control subjects (median [IQR] 73.6% [63.9%-80.7%] vs 62.0% [45.6%-73.8%], p = 0.004, Fig. 1, upper left panel; and 2.6% [1.1%-6.9%] vs 1.1% [0.6%-2.2%], p = 0.002, Fig. 1, upper middle panel). In addition, the proportion of IFN-γ-producing CD4$^+$ T cells was significantly higher in patients with COPD than in healthy control subjects (25.7% [21.2%-38.0%] vs 22.8% [15.6%-29.2%], p = 0.027, Fig. 1, lower left panel).

However, there was no difference in the proportion of IL-4-producing CD4+ T cells or in the ratio of IFN-γ-producing/IL-4-producing CD8$^+$ or CD4$^+$ T cells between the 2 groups (Fig. 1, upper right, and lower middle and right panels).

PERIPHERAL BLOOD T-CELL PROFILES: EFFECT OF SMOKING AND DISEASE SEVERITY

There was no significant difference in the proportion of IFN-γ- or IL-4-producing CD8$^+$ or CD4$^+$ T cells or in the ratio of IFN-γ-producing/IL-4-producing CD8$^+$ or CD4$^+$ T cells between smokers and ex-smokers. Neither was any difference among patients with mild, moderate, and severe COPD (Table 2).

CORRELATION BETWEEN T-CELL PROFILES AND CLINICAL AND INFLAMMATORY PARAMETERS IN PATIENTS WITH COPD

As shown in Table 3, the proportion of IFN-γ-producing CD8$^+$ T cells was correlated negatively with Kco and positively with eNO (Fig. 2). The proportion of IL-4-producing CD8$^+$ T cells was positively correlated with BMI and Kco. In addition, the ratio of IFN-γ-producing/IL-4-producing CD8$^+$ T cells was negatively correlated with BMI and Kco. There was no correlation between CD8$^+$ T cell profiles and other parameters.

The proportion of IL-4-producing CD4$^+$ T cells was negatively correlated with FEV_1/FVC, and the ratio

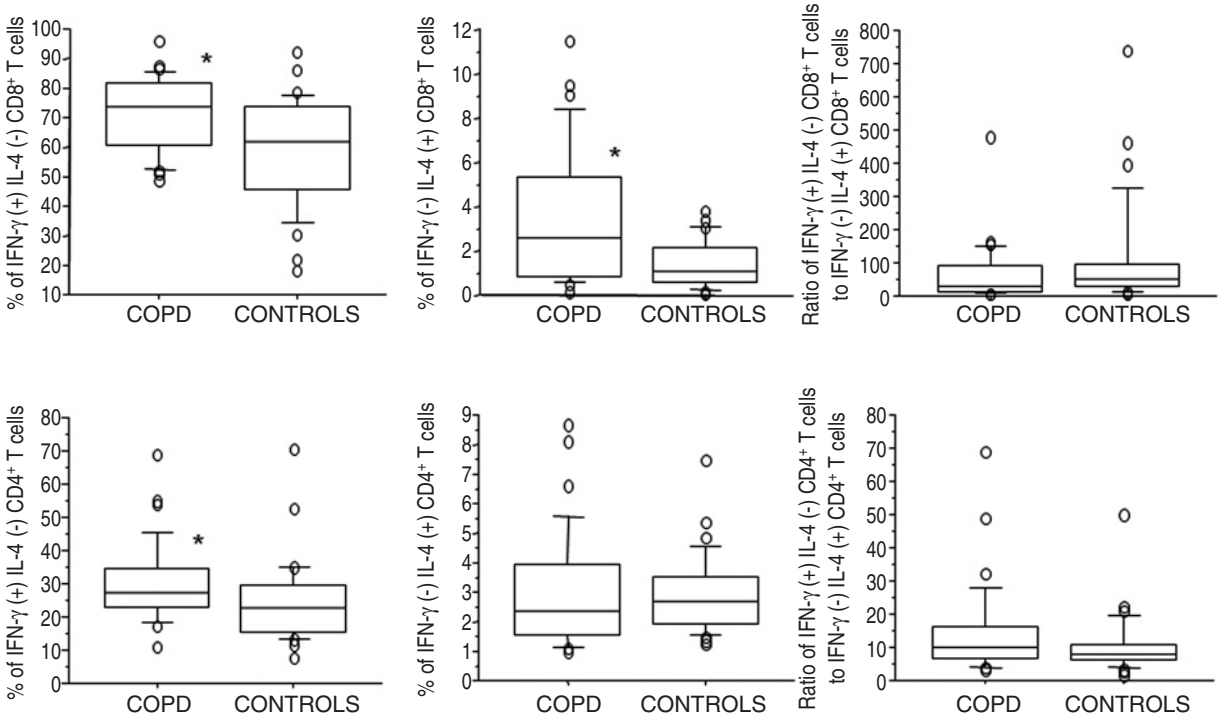

Fig. 1 The percentage of IFN-γ (+)/IL-4 (-) CD8+ T cells and IFN-γ (-)/IL-4 (+) CD8+ T cells, the ratio of IFN-γ (+)/IL-4 (-) CD8+ T cells to IFN-γ (-)/IL-4 (+) CD8+ T cells, the percentage of IFN-γ (+)/IL-4 (-) CD4+ T cells and IFN-γ (-)/IL-4 (+) CD4+ T cells, and the ratio of IFN-γ (+)/IL-4 (-) CD4+ T cells to IFN-γ (-)/IL-4 (+) CD4+ T cells obtained from peripheral blood after stimulation with PMA/ionomycin. Horizontal lines, boxes, whiskers, and circles represent median values, the interquartile range, 10th or 90th percentiles, and data apart from 10th or 90th percentiles, respectively. *$P < 0.05$.

Table 3 Correlations between CD8+ T cells and clinical and inflammatory parameters in patients with COPD †

	IFN-γ-producing CD8+ T cells	IL-4-producing CD8+ T cells	IFN-γ-producing/IL-4-producing CD8+ T cells
BMI	-0.33	0.42	-0.43
		$p = 0.023$	$p = 0.020$
Pack-years	0.33	-0.06	0.06
FEV_1	-0.10	0.08	-0.08
FEV_1/FVC	-0.11	0.04	-0.04
TLC	0.11	-0.15	0.14
FRC	0.10	-0.15	0.13
RV	0.22	-0.21	0.20
Kco	-0.45	0.47	-0.48
	$p = 0.033$	$p = 0.026$	$p = 0.025$
Sputum neutrophils	-0.13	0.06	-0.08
Exhaled NO	0.50	-0.30	0.32
	$p = 0.012$		
High sensitivity CRP	0.07	0.04	-0.02

BMI, body mass index; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; Kco, carbon monoxide transfer coefficient; NO, nitric oxide; CRP, C-reactive protein.

† Values are the correlation coefficient with statistical significance. If only a number is shown, it is not significant.

of IFN-γ-producing/IL-4-producing CD4+ T cells was positively correlated with FEV_1 and FEV_1/FVC (Table 4); however, the association was weak in view of each correlation coefficient value.

No correlation was found between CD4+ T cell profiles and other parameters.

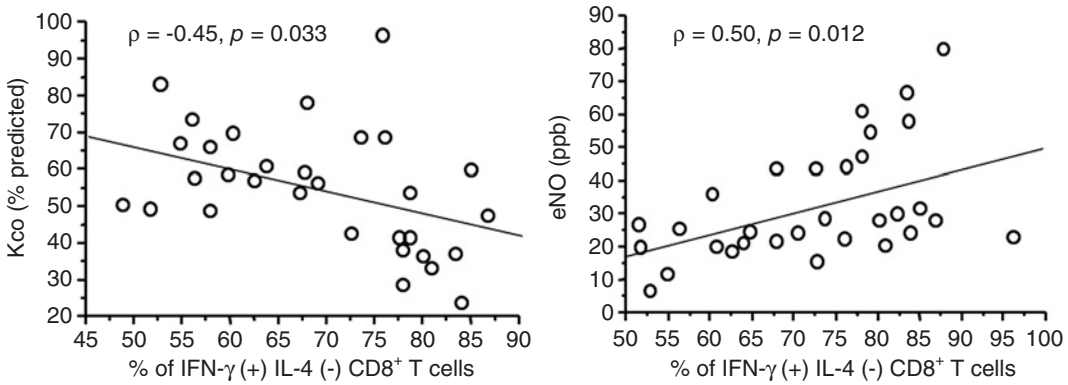

Fig. 2 Correlations between the proportion of IFN-γ (+)/IL-4 (-) $CD8^+$ T cells and transfer coefficient (Kco) and exhaled nitric oxide (eNO).

Table 4 Correlations between $CD4^+$ T cells and clinical and inflammatory parameters in patients with COPD †

	IFN-γ-producing $CD4^+$ T cells	IL-4-producing $CD4^+$ T cells	IFN-γ-producing/IL-4-producing $CD4^+$ T cells
BMI	-0.04	-0.04	0.04
Pack-years	-0.01	0.11	-0.11
FEV_1	0.08	-0.30	0.36
			$p = 0.049$
FEV_1/FVC	0.03	-0.37	0.39
		$p = 0.045$	$p = 0.033$
TLC	0.29	0.23	-0.04
FRC	0.11	0.19	-0.20
RV	-0.01	0.35	-0.37
Kco	-0.10	0.06	0.00
Sputum neutrophils	-0.00	0.11	-0.04
Exhaled NO	0.07	-0.10	0.13
High sensitivity CRP	-0.04	-0.13	0.12

BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; Kco, carbon monoxide transfer coefficient; NO, nitric oxide; CRP, C-reactive protein.

† Values are the correlation coefficient with statistical significance. If only a number is shown, it is not significant.

DISCUSSION

In this study we found a significantly higher percentage of IFN-γ-producing and IL-4-producing $CD8^+$ T cells in peripheral blood after PMA/ionomycin stimulation in patients with COPD compared with healthy control subjects. Considering the high proportion of IFN-γ-producing $CD8^+$ T cells, our results provide indirect support for Tc1 predominance in the lungs shown by an immunohistochemical study.[5] In addition, a higher proportion of IFN-γ-producing $CD4^+$ T cells was found in COPD patients than in control subjects.

The profiles of cytokine production in peripheral blood T cells in patients with COPD have been reported previously.[10,11] Barceló *et al*. showed no difference in the proportion of IFN-γ-producing or IL-4-producing $CD8^+$ T cells between patients with COPD and control subjects.[10] Majori *et al*. also showed no difference in the percentage of Tc1 or Tc2 cells[11]; however, a significantly increased proportion of IFN-γ-producing and a reduced proportion of IL-4-producing $CD4^+$ T cells were found in COPD patients compared with control subjects.[11] Th1 predominance in the latter report is consistent with our study; however, there are several differences in other results. Basically, there is no difference in methodology between these reports and our study. No subjects had received oral or inhaled corticosteroids in our study, whereas some of the patients studied by Barceló *et al*. were given inhaled corticosteroid. Overall, our patients had milder COPD as reflected by a median %FEV_1 of 63.5%, compared with 42.4% in the study reported by Majori *et al*. These could all be causes of the discrepancy.

Recently, Zhu *et al*. also reported an increase of Tc 1 and Tc2 in the peripheral blood in patients with COPD compared with lifelong nonsmoking, control

subjects.[27] Koch *et al*. found an increased expression of the Tc1-specific chemokine receptor CXCR3+ by blood CD8+ T cells in smokers with COPD compared with smokers without COPD and controls, and an increased expression of activated and cytotoxic effector CD8+ T cells in smokers with and without COPD compared with controls.[28] These findings suggest the possibility that the increase of Tc1 (and possibly Tc2) may be induced by smoking.

Recent studies have investigated cytokine production by bronchoalveolar lavage T lymphocytes and found an increased population of CD8+ cells differentiated into the Tc2 rather than the Tc1 profile in the lungs of patients with COPD.[10,12] In contrast, in a study reported by Tzanakis *et al*., there was a significantly lower proportion of Tc1 and Tc2 subtypes and a higher ratio of Tc1/Tc2 in induced sputum T cells in the patients compared with the control subjects, raising the possibility that airway T lymphocyte profiles might be different from those of peripheral blood.[13] Thus, a consensus has not been established and further studies should clarify whether Tc1 or Tc2 cells are predominant in the peripheral blood or lungs of COPD patients.

We found a negative correlation between the proportion of IFN-γ-producing CD8+ T cells and the ratio of IFN-γ-producing/IL-4-producing CD8+ T cells and Kco. Also, we found a positive correlation between the proportion of IL-4-producing CD8+ T cells and Kco. These results suggest that Tc1 cytokine production might be parallel and Tc2 cytokine production might be the reverse of the degree of gas exchange abnormalities. We also found a positive correlation between the proportion of IL-4-producing CD8+ T cells and BMI, which was significantly higher in patients with mild COPD compared with severer patients. Overall, it is possible that Tc2 cells play a protective role against Tc1 cells in the inflammatory process of the disease. Other investigators have also reported a relationship between T-cell profiles and pulmonary function. Barceló *et al*. found a negative correlation between the proportion of IL-4-producing CD8+ bronchoalveolar lavage T cells and FEV_1,[10] whereas Tzanakis N *et al*. reported a positive correlation between the ratio of IFN-γ-producing/IL-4-producing CD8+-induced sputum T cells and FEV_1.[13] Kim *et al*. found that COPD patients with normal diffusing capacity (DLCO/VA = 80%) had a significantly higher proportion of CD8+ T lymphocytes than healthy smokers but they did not study the cell types, Tc1 or Tc2.[29] The selective recruitment of cells out of the blood into the tissue, and out of the tissue into the lumen may cause differences between these compartments.

We also found a positive correlation between the proportion of IFN-γ-producing CD8+ T cells and eNO. Previous studies demonstrated a negative correlation between exhaled NO levels or induced sputum neutrophil counts and FEV_1,[14-16] while recent studies have shown that eNO is not associated with the severity of COPD[30] and that various factors, including eNO, airflow limitation, and sputum eosinophils and neutrophils are separate and largely independent components of COPD pathophysiology.[31] In general, exaggerated active oxygen intermediates are thought to convert NO to nitric metabolites, thereby reducing the airway NO levels in COPD.[32] In addition, NO is induced from epithelial cells by IFN-γ.[33,34] Taken together, our findings do not imply a direct relationship between Tc1 cells and airway inflammation.

The proportion of IFN-γ-producing CD4+ T cells was higher in COPD patients than control subjects, but there was no correlation between clinical or inflammatory parameters. Also, there was a positive correlation between the proportion of IL-4-producing CD4+ T cells and FEV_1/FVC, but the association was weak. These findings suggest that CD4+ T cells might also play a role in the pathogenesis of COPD, but the mechanisms are unclear.

We did not study the proportions of CD4+ or CD8+ T cells in peripheral blood. There are conflicting results about this in the literature. No increase or decrease of CD4+ (range: 35% to 70%) or CD8+ T cells (range: 20% to 30%) compared with controls has been reported,[10,11,27] whereas an increased proportion of CD4+ T cells[27] or decreased CD8+ T cells[28] has been found.[11]

In conclusion, both Tc1 and Tc2 cell proportions are higher in the peripheral blood of stable COPD patients than in healthy controls. The proportion of Tc1 cells is parallel to the degree of gas exchange abnormalities, while the proportion of Tc2 has an opposite degree of gas exchange abnormalities. It is suggested that Tc1 cells have a detrimental role and that Tc2 cells have a protective role in the progression of COPD.

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